

**Progress report, January-August 2009**

**IDENTIFICATION OF THE GENOMIC LESIONS UNDERLYING RICHTER'S SYNDROME**

**Candidate**

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## **Progress report**

CLL is the most common leukemia in the Western world. Transformation of CLL to an aggressive lymphoma, usually a diffuse large B-cell lymphoma (DLBCL) (Richter's syndrome, RS) occurs in 5-15%. RS is more aggressive and chemo-refractory than de novo DLBCL. The reasons are still unknown.

With the aim of identifying the genomic lesions underlying RS pathogenesis, we have set up a large international network to collect material. As explained in the original project proposal, we planned to collect DNA of at least from 150 cases to be analyzed for the presence of genomic lesions using the Affymetrix GeneChip Genome-Wide Human SNP Array 6.0 and for their immunogenetic status study.

In the period January-August 2009, we have collected a total of 276 DNA samples from 20 Centers and the collection is still in progress. So far, 154 cases are from paraffin embedded formalin-fixed (FFPE) tissue and 122 from fresh frozen biopsy material. One third of all cases comprises both CLL and RS-phases from the same patient. Clinical data database is ongoing.

Regarding the characterization of the immunogenetics (IgHV usage) and chromosomal aberrations of RS, a first group of FFPE samples have already been analyzed. An abstract has been submitted to the next meeting of the American Society of Hematology (ASH), and a full manuscript is in preparation.

Briefly, the data showed:

- i) High prevalence of TP53 mutations in RS (Figure 1)
- ii) RS-DLBCL has specific profile of genetic lesions when compared to de novo DLBCL (Figure 2)
- iii) Differently from somatic mutations, TP53 deletion is not a common mutational event
- iv) TP53 mutations predict survival in RS (Figure 3)

A manuscript on the pilot project of genome wide DNA profiling on 13 samples of RS, eight samples of RS-CLL and, for comparative purposes, 48 de novo DLBCL.

We concluded the following :

- i) By comparing genomic alterations in RS versus de novo DLBCL, RS were characterized by a significantly lower prevalence of deletions of 6q, and a higher frequency of deletions of 10q25.2 and at 20p.
- ii) A frequent aberration in RS was the gain of the 13q13.3-qter region, containing *MIR-17-92*, a cluster of microRNA, which was acquired at the time of transformation and absent in the pre-existing CLL phase. *MIR-17-92* is known to be involved in lymphomagenesis by interacting with the *c-MYC* pathway.
- iii) Gain of the *MIR17-92* cluster in our RS series was found to be matched with a recurrent pattern of genetic lesions characterized by the concomitant gain of *c-MYC* (8q24) and loss of *TP53* (17p), and mutually exclusive with *c-MYC* translocations. As a whole, up to 50% of RS seem to acquire at least one genetic lesion affecting *c-MYC* pathway.

During these months 266 CLL samples have also been analyzed with Affymetrix Human Mapping 6.0 arrays. The first data have been sent as an abstract to the next ASH meeting. The application of high resolution arrays on a large series of CLL samples has shown new small interstitial deletions and identified genomic lesions associated with a different outcome. Of interest, obtained genomic profiles will be used as a comparison with the CLL phases of RS in the current project.

**In conclusion:**

**First year:**

- i) A large part of RS cases have been already collected.
- ii) Immunogenetic study is in process and some interesting preliminary data are ready for publication.
- iii) Sufficient amount of fresh frozen material allows us to initiate the high-throughput analysis by Affymetrix GeneChip Genome-Wide Human SNP Array 6.0

confidential data, September 2, 2009

**Second Year Milestone will be, as already planned in the original Planned Translational research program:**

- i) Collection of the clinical data
- ii) Analysis of DNA profiles
- iii) Combination of DNA profiles with clinical, pathological and other available data  
(gene expression profiling)
- iv) Preparation of the manuscript and submission of an abstract

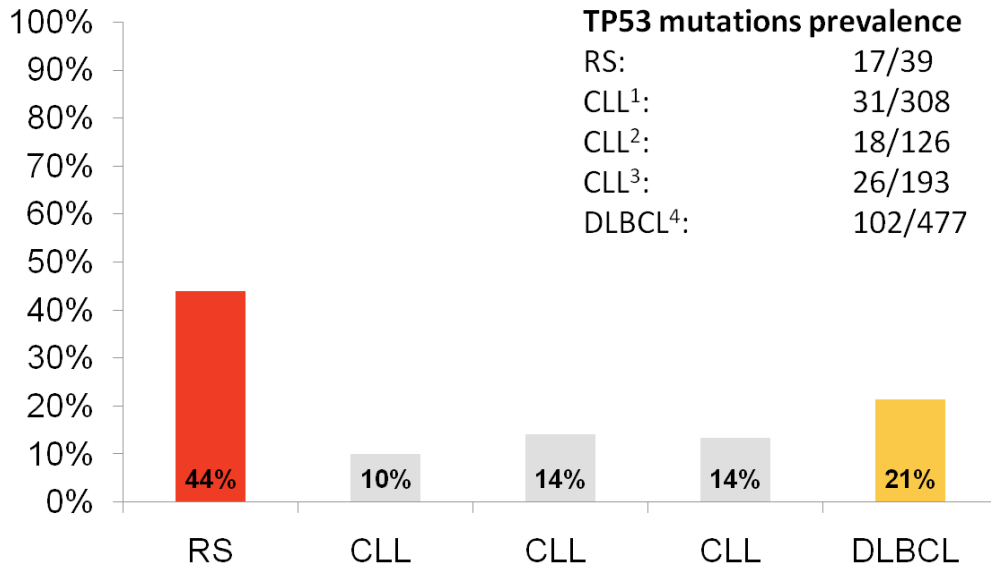


Figure 1. Prevalence of TP53 mutation in RS

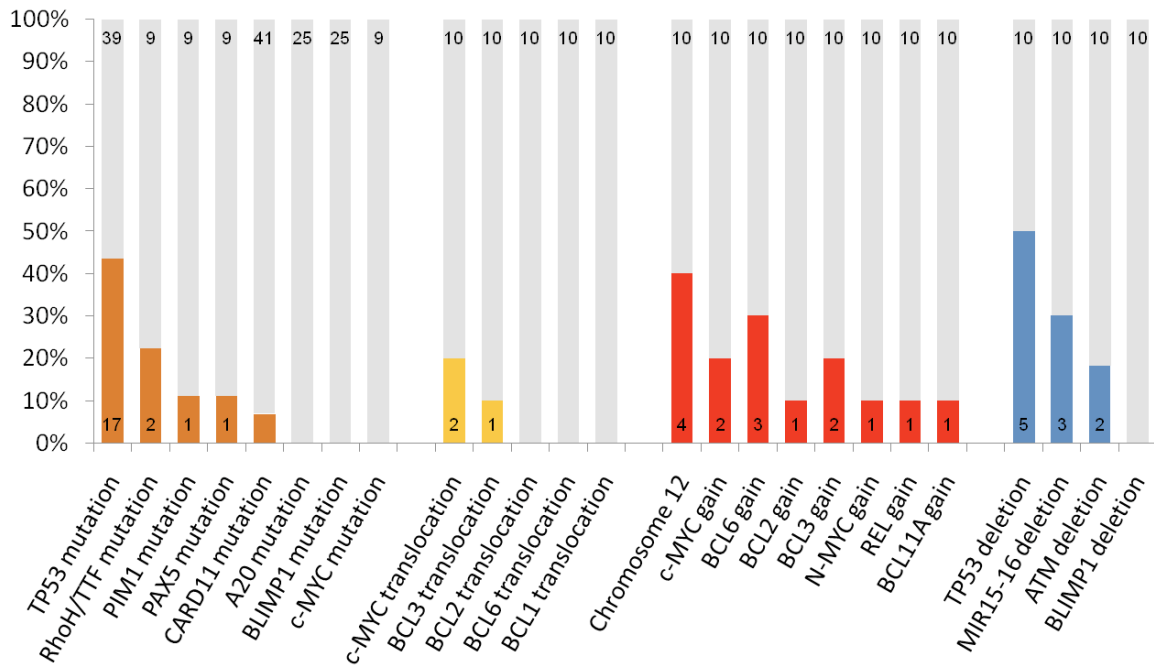


Figure 2. Genetic lesion of RS-DLBCL

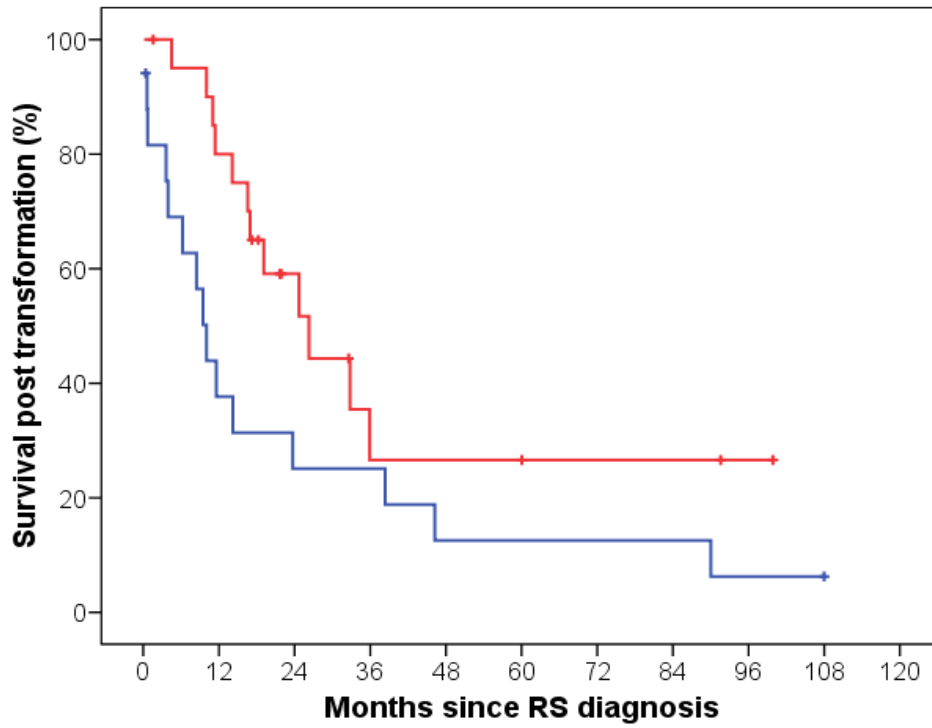


Figure 3. TP53 mutational status predict survival in RS